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A STUDY OF BAT RABIES IN OHIO

A Thesis

Presented in Partial Fulfillment of the Requirements
for the Degree Master of Science

by

Roger Alan Krogwold, D.V.M.

The Ohio State University
1977

Approved by

Adviser
Department of Veterinary
Preventive Medicine

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DEDICATION

This thesis is dedicated to my wife, Linda, whose assistance, patience, support, and understanding aided me in completion of this endeavor.

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INTRODUCTION

During 1976, the Ohio Department of Health reported 23 bats positive for rabies (47). This appeared to be an unexpectedly high number. Of the 23 positive bats, only 9 were identified as to species (41,51). Eight (5 Myotis lucifugus and 3 Eptesicus fuscus) of these were colonial-living species. The other was Lasionycteris noctivagans, a solitary-living species.

Bats, for rabies examination, were submitted to the State Laboratory by local health departments and individual citizens. These people were aware of bat rabies and usually submitted bats for the following reasons:

1. A human or pet was directly exposed to a bat by bite or contact.
2. The bat was sick or injured when found.
3. The bat was captured after it entered a room or building and was unable to find its way out.

In all of these cases, an unusual situation brought the person and bat into confrontation. Therefore, these bats were obviously not a random sample, but probably represent a biased sample of the normal bat population. Since the observation of 23 bats reported positive for rabies in 1976 might have been due to a higher prevalence of rabies in the bat population, the present

study was undertaken to analyze available data on bat rabies in Ohio and to sample the colonial bat population in Ohio to determine if they were infected with rabies virus. Colonial bats were sampled because they made up the majority of identified rabies positive bats during 1976. Also, colonial species were more easily captured than solitary-living species, thus aiding in collection of sufficient bats for this study.

The major objectives of this study were:

1) To retrospectively analyze the temporal and spatial distribution of bat rabies cases reported by the Ohio Department of Health, and

2) To conduct laboratory tests on a sample of the wild bat population to determine if an epizootic of bat rabies exists in Ohio.

REVIEW OF LITERATURE

Bats were first associated with rabies in animals in Brazil during the early twentieth century. The etiology of the Brazilian disease was initially unknown, but was thought to be rinderpest. Haupt and Rehaag (33) investigated the epizootic that was occurring in the population. They noted that the disease was similar to rabies, but a means of rabies transmission was not established. They concluded that terrestrial animals were not responsible for transmission of rabies because the disease had crossed a river which no terrestrial animal could cross. Some of the local villagers believed that the disease was transmitted by bats. Supportive evidence for this was demonstrated in 1914 when a calf died of rabies after being exposed to the bite of a bat. The calf was kept in a stable and was never let outside. The door of the stable was left open one day and apparently a bat entered. The door was closed, but later that day the farmer's wife responded to the calf's restlessness. She noticed that a bat was biting the calf. She killed the bat and her husband submitted it to Haupt and Rehaag for examination. The bat showed no Negri bodies on examination, but there wasn't sufficient brain material for animal inoculation. The calf died 27 days after being

bitten by the bat and on examination of the medulla, Negri bodies were identified.

In 1916, Rehaag (33) demonstrated Negri bodies in the brain of a leaf-nosed, fruit-eating bat (Artibeus lituratus). He inoculated brain material into a guinea pig and a rabbit and observed Negri bodies in the brains of these animals when they died 13 and 15 days later, respectively. This was the first isolation of rabies virus from a bat. He concluded that rabies, transmitted by bats, was causing the epizootic of rabies in Brazil's cattle and horses.

There remains some question concerning the species of the bat. Rehaag identified this bat as Artibeus lituratus, a leaf-nosed bat, while in earlier identification of leaf-nosed bats, Haupt had claimed that these were vampire bats (Desmodus rotundus).

The first rabies positive insectivorous bat was reported by Pawan in 1932 (43). The bat, Diclidurus albus, is commonly known as a white bat and was found resting on a cow during daylight hours. The diagnosis was based on animal inoculation of brain material from the bat into 2 rabbits and 4 guinea pigs. Paralysis, death, and demonstration of Negri bodies proved the presence of rabies virus and that insectivorous bats could carry rabies.

The diagnosis of rabies in bats did not initially appear to be of great concern to the United States. Species of bats found positive for rabies were not indigenous to the United States. Therefore, no attempts were made to isolate rabies virus from bat species native to this country (55). Investigators were able, in 1951, to experimentally infect 2 very common bat species of the United States. The species of bats were the big brown bat (E. fuscus) and the little brown bat (M. lucifugus) (45). Even with this evidence, no investigations were undertaken to determine if naturally infected bats were present in the United States.

In June 1953, however, a Florida yellow bat (Dasypterus floridanus) attacked a young boy during daylight hours. His father, having heard of rabid vampire bats, submitted the bat to the Tampa Regional Public Health Laboratory for evaluation. The laboratory identified Negri bodies in the bat's brain and in the brains of mice that died following inoculation with the bat's brain (60).

Other states followed with laboratory diagnoses of rabies in bats. Pennsylvania reported a rabid hoary bat (Lasiurus cinereus) in 1953 (64). The bat had landed on a woman and bit her. After submission of the bat to the state laboratory and finding it positive for rabies, the

bat carcass was incinerated without positive species identification. The woman bitten by the bat, her spouse, and a game protector, who took the bat to the laboratory, established the bat's identity after examining bat skins from a local museum.

Texas also reported its first cases of rabid bats in 1953 (58). One Mexican free-tailed bat (Tadarida brasiliensis mexicana) and a pooled sample of 1 T. b. mexicana and 1 cave myotis (Myotis velifer) were found to be positive by mouse inoculation. These were the first colonial bats found infected in the United States.

California followed with a rabid insectivorous bat in 1954. The species was again T. b. mexicana (27). Montana also reported a rabid bat, E. fuscus, during 1954 (6).

These 5 states represented widely scattered areas in the United States where rabid bats were present. Although not discovered until 1953, it is thought that rabies existed in the bat population of the United States before this time. Evidence to support this was given by Sulkin and Greve in 1954 (56). They retrospectively reported that a case of human rabies occurred during 1951 in Texas. The woman affected had been bitten by a bat 16 days prior to her onset of illness. She died 5 days later and histopathologic examination confirmed rabies. The bat was

not retained for rabies examination, but no other known animal bite exposure had occurred. Based on the history of the case and the histopathologic findings, rabies infection in the woman was attributed to the bite of the bat.

By 1967, every state of the continental United States had reported at least 1 case of bat rabies. During that year, 414 rabies positive bats were reported in the United States (55). Although the number of reported cases steadily increased from 1953 to 1967, workers have demonstrated that the per cent positive of those submitted for testing has shown very little change, indicating that the prevalence of rabies in the bat population has remained relatively constant (17).

Presently, the remaining 2 states have not yet reported any cases of rabid bats. Sulkin (53) reported that Hawaii is free of the disease in every species of animal. Alaska has a very low bat population and has made no systematic effort to demonstrate rabies in bats. Evidence suggesting that Alaska might have rabies in its bat population has been provided by the isolation of rabies virus from 4 bats in British Columbia during 1957 and 1958. The bats were 2 E. fuscus, 1 M. lucifugus, and 1 silver-haired bat (L. noctivagans) (2).

Shortly after Florida's first rabies virus isolation from a bat, several studies were launched in various states to determine the prevalence of rabies in the bat population. The Florida State Board of Health conducted a survey during 1953 in which Fresh Water Fish and Game officials shot bats in flight. Most of these bats were solitary-living species, though groups of colonial bats were also obtained by the researchers. The total number of bats collected in this study was 384, of which 6 were positive for rabies by mouse inoculation and Negri body examination. Four of the rabid bats were from the immediate area of Florida's first rabid bat case. All of the rabies positive bats were from the 208 non-colonial species collected. Two and nine-tenths per cent of the non-colonial bats were rabies positive or 1.6% of the total bats collected (48,60).

The Florida State Board of Health also conducted a greatly expanded survey between 1953 and 1956. Again, people shot bats in flight, but they also gathered numerous bats from several roosting sites. Bats exhibiting Negri bodies were tested individually in mice, while negative bats were tested by pooling the brains from 3 bats for mouse inoculation. There were 8 rabies virus isolations from 3969 colonial bats and 18 from 1499 solitary-living bats examined. The prevalence rates were

0.2 and 1.2%, respectively, or an overall prevalence rate of 0.48% (49).

Sullivan et al. (58), in a 1953 Texas study, collected 151 T. b. mexicana, 42 M. velifer, and 7 eastern pipistrelles (Pipistrellus subflavus). Two T. b. mexicana were rabies positive by mouse inoculation and Negri body examination. Brain tissue from 1 of the T. b. mexicana was inadvertently mixed with the brain tissue of a M. velifer and the pooled sample was positive by mouse inoculation. Since the M. velifer was negative by Negri body examination, it was concluded that the T. b. mexicana was positive, while the M. velifer was questionable but probably negative. The authors remarked that both rabies positive T. b. mexicana behaved abnormally at time of collection, but they did not mention if any of the rabies negative bats showed abnormal signs. In this sample, 1% of the bats examined were rabies positive or 1.3% of the T. b. mexicana.

Another Texas study was conducted from 1954 to 1956. Burns et al. (11) found that 2 of 26 D. rotundus from Mexico, 20 of 186 T. b. mexicana, 1 of 10 desert pallid bats (Antrozous pallidus), and 2 of 6 red bats (Lasiurus borealis) were positive by mouse inoculation of brain suspension. These would correspond to prevalence rates of 7.7, 10.8, 10, and 33%, respectively, for the species

sampled. This study included D. rotundus. Although not found in the United States, D. rotundus' range extends into the Mexican states bordering Texas. Also noted in this study was the inconclusiveness of the Negri body test in bats, which detected only 17% of the positive bats.

A third Texas study captured 329 T. b. mexicana. Pooled samples were made from 294 bats, while 35 bats were tested individually. None of the individual bats tested were rabies positive, but 12 of 63 pooled samples were positive. Minimum rabies prevalence would have been 3.6% in this study (25).

Enright et al. (27), in a California survey, found 1 positive T. b. mexicana in 211 bats of 14 different species. Mouse inoculation and serum neutralization were used to detect rabid bats which showed a prevalence rate of only 0.45%. The particular number of each species or comments on the health of the captured bats were not included in the article.

The first rabid Ohio bat was found in a study done by Tjalma and Wentworth in 1955 (59). They collected 63 E. fuscus from a hibernating colony. Pools of 3 to 4 bats were inoculated into groups of 4 mice. One group later showed signs of rabies and 2 mice from this group were Negri body positive. Serum-virus neutralization techniques further proved that rabies virus was present in

at least 1 of the bats in the pooled sample.

Negative surveys have also been recorded. A survey in Montana showed no positives in a sample of 121 roosting bats (5). Verts and Barr (61) found no rabies positive bats in a sample of 559 bats taken during 1957 to 1958 in Illinois. They checked for rabies by pooling brains of 3 to 5 bats and inoculating suspensions of these intracerebrally into mice. The species included in this study were M. lucifugus, E. fuscus, P. subflavus, and L. borealis. Richardson et al. (46) found no positives in 218 colonial bats taken in Georgia. Another survey found no positive bats in 218 sampled from Massachusetts during 1958 (23).

A New England study found 5 rabies positive bats among 514 normal bats examined or 0.97% positive. This study was the first to use the fluorescent antibody (FA) technique for rabies diagnosis. E. fuscus accounted for 119 bats in the study and M. lucifugus made up the remaining 395 bats submitted. The prevalence rates were 2.5 and 0.5%, respectively, for these 2 species (30).

In an Arizona study, Dean et al. (24) reported 13 bats positive in a sample of 552 bats. Various species were represented in this study. The prevalence rate of rabies infected bats was 2.4%.

Constantine (22) conducted a large New Mexico survey from 1955 through 1962. Mouse inoculation, examination for Negri bodies, and the FA test were used to diagnose rabies. A prevalence rate of 0.3% was recorded from a sample of 2844 normal T. b. mexicana.

A summary of these studies is given in Table 1 to list bat species, number collected, number positive for rabies, and per cent positive. Only healthy bats from these surveys are included. Studies using pooled samples are included in this summary, but positive pools are listed as individual bats. Since the minimum number of positive bats in a positive pool is one, the number and per cent positive are minimums.

In all, 40 species of bats are known to inhabit the United States. All are insectivorous except 3 which are nectar suckers. Of these 40 species, only 11 have ranges extending into Ohio and all of these species are insectivorous. One of these is the Florida free-tailed bat (Tadarida brasiliensis cynocephala), which has been reported in Ohio and whose range extends into the southern tip of Ohio, according to Barbour and Davis (4). This species is extremely rare in Ohio.

Six of the 11 species are quite rare. These are Keen's bat (Myotis keeni), the small-footed myotis (Myotis leibii), the Indiana bat (Myotis sodalis), the evening bat

(Nycticeius humeralis), the silver-haired bat (Lasionycteris noctivagans), and the hoary bat (Lasiurus cinereus) (4). One of these, M. sodalis, is on the list of endangered species (12).

The other 4 bat species in Ohio are: E. fuscus and M. lucifugus, which are abundant colonial bats; P. subflavus, a colonial species, but less abundant; and L. borealis, a fairly abundant solitary species (4).

These 4 species are also diverse in their migratory habits. L. borealis is highly migratory, traveling several hundred miles during spring and fall. E. fuscus is considered to be a permanent resident and usually hibernates within a few miles of its summer home. M. lucifugus is also known to be a permanent resident, but they have been known to migrate 290 miles to seek a cave or abandoned mine for hibernation. P. subflavus' migration patterns have been poorly studied, but in those studies conducted, these bats have migrated less than 50 miles (4).

Serum neutralizing antibody for rabies virus was checked in only 4 studies. The first study, conducted by Burns and Farinacci in 1954 (10), used 200 serum samples grouped into 35 pools. All serum samples were from T. b. mexicana. Their results showed 65% of the pools positive, 9% equivocal, and 26% negative in protection against 100

mouse LD_{50}'s of CVS strain of rabies virus. Since this was a pooled serum study, only an estimate can be made of how many individual bats had rabies neutralizing antibody. If 1 bat in each positive pool carried antibody, then at least 23 bats in the study would have been considered positive or 11.5%.

A second study using the same procedure was conducted by these authors in 1954 and 1955. Three to 12 bats were combined for each pooled serum sample. Sixty-one per cent of the T. b. mexicana, 74% of the T. b. cynocephala, and 24% of the M. velifer serum pools contained neutralizing antibody against the CVS strain. Minimum prevalence rates of bats carrying neutralizing antibody against rabies virus would have been 9.7, 14.9, and 4.3%, respectively. Three other species included in the study (A. pallidus, L. borealis, and D. rotundus) were negative for rabies neutralizing antibody (11).

No positive serum samples were reported in a Florida survey. The same technique, as used by Burns and Farinacci (10), was employed. Serum samples from 245 T. b. cynocephala were combined into 49 pools for this survey (49).

The only study using individual serum samples from bats was done by Constantine and his co-workers in 1968 (22). All bats sampled were T. b. mexicana. Two hundred eighty-three of 1305 bats collected (21.7%) had rabies

neutralizing antibodies.

In an effort to delineate how the bat could serve as a persistent reservoir of rabies virus in nature, Sulkin (57) experimented with intramuscular inoculation of rabies street virus (Thompson strain) into T. b. mexicana. His results showed 11 of 137 bats (8.0%) with rabies virus in their interscapular brown fat, as compared to 28 of the 137 bats (20.4%) with brains positive for rabies. Determination of virus presence was by mouse inoculation.

Isolation of rabies virus from brown fat of naturally infected bats was achieved by Bell and Moore in 1960 (7). They recovered rabies virus from the brown fat of 1 M. lucifugus and 1 E. fuscus that had rabies positive brains. Subsequently, they isolated rabies virus from the brown fat of 5 additional bats.

Sulkin (54) conducted a survey of brown fat from 500 bats, but was unable to recover rabies virus from any of the bats. Dean and his co-workers (24) had given Sulkin these bats from their Arizona study. Ideal storage conditions may not have been maintained.

MATERIALS AND METHODS

Immunization of the Investigator

The investigator was immunized against rabies with a series of three inoculations of duck embryo vaccine (DEV) in 1969 and 1970. An annual booster was given each year from 1972 to 1976. Rabies antibody titer was measured prior to receiving the last inoculation. A 1:15 titer was recorded by the RFFIT method at the Center for Disease Control in Atlanta, Georgia.

People offering to assist the investigator were warned of potential rabies exposure before they assisted in any collection of bats.

Collection of Bats

Healthy bats were obtained from several locations in the state. Collections were made from 9 September 1976 through 27 June 1977.

County and city health departments, Ohio Department of Natural Resources, Ohio Department of Health officials, and pest exterminating agencies were contacted to gain information on potential bat colonies. Property owners often contacted these agencies to get information on how to rid their buildings of bats. Locations for potential collections were examined by local health department personnel or the investigator to determine if a bat colony

was present and what equipment would be necessary to capture the bats. In isolated instances, single bats that had been captured in a building were brought to the investigator. No bats captured in this study had any known history of biting humans or pets.

Protection for the investigator was achieved by wearing heavy trousers, a long-sleeved shirt, boots, cap, and respirator. Leather gloves were worn during all collections and for handling of living bats. Twelve-inch thumb forceps were used to retrieve hiding bats from cracks and crevices. A mist net was used in two locations where bats were not accessible for hand collecting. These latter 2 collections had to be made at dusk.

Captured bats were confined in glass jars, collapsible fish traps, or mosquito traps and transported to the laboratory. Use of glass jars and fish traps were discontinued because of asphyxiation in the former and escape from the latter.

Processing of Bats

Bats were processed immediately after returning to the laboratory or refrigerated overnight. Identification of the bats, according to species, was done using Barbour and Davis' book, Bats of America (4). Verification of species was made by Ms. Margaret Parsons, Head of the Vector Borne Disease Unit, Ohio Department of Health.

Carbon dioxide was used to kill bats in the laboratory. To process each bat, the hair over the head and shoulder area was moistened with 70% isopropyl alcohol. A dorsal midline skin incision was made rostrally from the occipital protuberance of the skull to the nose. Caudally, the incision was extended to the last thoracic vertebrae. The skin was reflected laterally to completely expose the interscapular brown fat and the dorsal skull surface with its associated musculature.

The interscapular brown fat was grasped with sterile thumb forceps and cut free with sterile scissors. A portion, approximately 1/16 inch in diameter, was cut from the ventral surface of the brown fat and used to make a squash smear between 2 microscope slides. The smears were allowed to air-dry for 1/2 hour before being fixed. The remaining brown fat was placed in an individual vial and frozen at -70°C.

To extract the brain, the calvarium was first removed. A sterile spatula was used to scoop the brain from the cranial cavity. The caudal 1/3 of the brain was cut off and an impression smear was made on each of 2 microscope slides from the brain's cut surface. The smears were allowed to air-dry for 1/2 hour. Remaining brain tissue was placed in an individual vial and frozen at -70°C.

After air-drying, the brain and brown fat smears were fixed by submersing in cold acetone (-20°C) for 12 to 20 hours. The slides were then air-dried in the -20°C freezer before being transferred to the -70°C freezer. Slides remained in the -70°C freezer until used for staining.

Negative Controls

Eight normal mice, 13 to 14 grams, CF1 strain*, were sacrificed for preparation of normal mouse brain and brown fat smears. Dissection of the mice and fixing and storage of the smears was done in the same manner as for the bats. About 30 to 40 impression smears were made from each mouse.

Positive Controls

Modified live virus rabies vaccine** was diluted with the company's supplied diluent. Further dilution was achieved with sterile triple distilled water to obtain a dilution of 5×10^{-2}. This dilution contained approximately 75 mouse LD_{50}'s per 0.03 ml.

Nine healthy laboratory mice, 13 to 14 grams, CF1 strain, were used for intracerebral inoculation of the diluted rabies vaccine. A 26 gauge, 3/8 inch needle, on a

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* Mid-Continent Research Animals, Inc., Shawnee, Kansas.

** Jen Sal ERA strain: Rabies Vaccine, Modified Live Virus, Porcine Tissue Culture Origin, High Cell Passage, Street Alabama Dufferin Strain.

tuberculin syringe, was thrust through the calvarium at a point 2 mm. to the right of the midline and at the apex of an angle whose sides crossed the right eye and right ear of the mouse. The needle was inserted 2 to 3 mm. into the brain and 0.03 ml. of the diluted rabies vaccine was injected.

Mice dying within 96 hours post-inoculation were destroyed. The remaining mice were sacrificed when moribund (9 to 10 days post-inoculation) for harvesting of brain tissue and brown fat. Twenty brain impression smears and 2 brown fat smears were made from each mouse and fixed and stored in the same manner described for the bats.

Preparation of Anti-rabies Conjugate, Staining of Smears, and Mouse Inoculation

The method described here is taken largely from the Center for Disease Control's laboratory manual entitled: Course 8260-C, Laboratory Methods for the Detection of Rabies (13).

Healthy laboratory mice were sacrificed and their brains harvested into a pre-weighed petri dish. After weighing, brains were transferred to a sterile glass vial and frozen at -70°C for at least one day.

The brains were removed from the freezer and warmed to 5°C. Four ml. of egg yolk diluent (20 ml. egg yolk

from a 6 day old embryonated egg and 170 ml. phosphate buffered water, pH 7.7) were added for each gram of brain tissue. The mixture was homogenized in a Ten Broeck grinder. Centrifugation of the suspension was accomplished in a refrigerated (5°C) centrifuge for 15 minutes at 330 x g. The supernatant, which was the 20% normal mouse brain suspension, was drawn off with a pipette and dispensed in 4.5 ml. aliquots into sterile glass vials. These vials were frozen at -70°C until needed for staining.

Fluorescein labeled anti-rabies globulin* was diluted with 5 ml. sterile triple distilled water. Three-tenths ml. of the diluted anti-rabies globulin was placed into each of 16 sterile glass vials. These vials were frozen at -70°C.

At the time of staining, 1 vial containing 0.3 ml. of fluorescein labeled anti-rabies globulin and 1 vial containing 4.5 ml. of 20% normal mouse brain adsorbing suspension were removed from the -70°C freezer and allowed to thaw. The contents of the 2 vials were combined and gently mixed to form the anti-rabies conjugate.

A positive and negative brain impression smear and a negative brown fat smear were stained for every 10 to 12

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* Baltimore Biologics Laboratory, Becton, Dickinson and Company.

field specimens. Slides to be stained were removed from the -70°C freezer and warmed to room temperature. Any condensation was allowed to dry before proceeding with staining. A 15 mm. diameter area of each smear was delineated with a wax pencil. Conjugate was applied to the ringed area and spread with an applicator stick. A plastic slide box with a water-soaked paper towel fixed in the cover was used to provide a moisture saturated atmosphere. Slides, with conjugate on them, were placed in the slide box and incubated at 37°C for 35 minutes. Slides were washed in a non-circulating phosphate buffered saline bath, pH 7.7, for 10 minutes. A distilled water rinse followed and the slides were air-dried. Buffered glycerine mounting medium, pH 8.5, was applied to the stained area and a coverslip placed over it.

Frozen brain and/or brown fat to be prepared for mouse inoculation, was placed in a pre-weighed Ten Broeck grinder. Refrigerated (5°C) horse serum diluent, made up of 4 ml. filtered horse serum, 186 ml. phosphate buffered water (pH 7.7), and 1.9 ml. gentamycin (1:10 dilution), was added to achieve a 15% mixture. After homogenizing, the suspension was centrifuged at 160 x g for 7 minutes in a 5°C centrifuge. The supernatant was extracted with a tuberculin syringe and 0.03 ml. was inoculated intracerebrally into each mouse of the designated group.

Mice used weighed 13 to 14 grams and were CF1 strain. Any mice dying prior to 48 hours post-inoculation were destroyed. Any mice dying after 48 hours post-inoculation were examined for rabies by the FA technique.

Microscopy

A Zeiss Standard 14 microscope with IV FL vertical illuminator was used to read all stained slides. The objective used was a Neofluar 40/0.75 without oil immersion. The power supply was transformer-regulated to control voltage at 12 volts. The light source was a 100 watt, 12 volt halogen bulb. The Zeiss light filter system used was Blue Excitation, FITC, Auramine. Filters included in this system are a 510 reflector, 440-490 nm. primary filter, and 520 nm. secondary filter.

Statistical Analysis

A one-tail t-test, using proportions, was the method of choice for analyzing data in this study. Differences between observed and expected results were statistically significant if $p < 0.05$ and of borderline significance if $0.05 < p < 0.10$.

RESULTS

The total number of bats submitted to the Ohio Department of Health Laboratory for rabies examination each year and the number of rabies positive bats reported each year for the period 1941 through 1976 are presented in Table 2. Species and number of bats positive for rabies in Ohio have been 22 E. fuscus, 9 M. lucifugus, 5 L. borealis, 3 L. cinereus, and 1 L. noctivagans (Table 3). Unidentified bats accounted for the remaining 47 positive bats. The most common reasons for not identifying bats were mutilation and decomposition of the specimens (41,50).

There were 87 bats diagnosed rabies positive in Ohio from 1955 through 1975. Two of the positive bats were not from regular submissions to the State Laboratory. These 2 bats were from surveys of bat colonies in Ohio. The first of these was in 1955 and it was the first rabies virus isolation from a bat in Ohio. The virus isolation was made by Tjalma and Wentworth (59) from a sample of 63 E. fuscus taken from a colony in Franklin County. The second was in a sample of 35 bats taken from a colony of M. lucifugus in Stark County during 1958 (39). Neither of these bats is included in the statistical analysis because these bats were from random samples rather than regular submissions to the State Laboratory. The 7 rabies

positive bats reported for 1967 have also been omitted from the following statistical analysis. These bats were among regular submissions to the State Laboratory, but the total number of bats submitted in 1967 was not known.

During the period, 1956 through 1975, 2468 bats were submitted to the State Laboratory for rabies examination. Seventy-eight (3.16%) of these bats were rabies positive. The total number of bats submitted in 1976 was 488, of which 23 (4.71%) were rabies positive. The 1976 ratio (4.71%) is of borderline significance ($0.05 < p < 0.10$) when statistically compared to the ratio (3.16%) for the time period 1956 through 1975.

The geographic distribution of rabies positive bats diagnosed in Ohio from 1955 through 1975 is shown in Figure 1 and the counties of origin of rabid bats for each year are presented in Table 4. Before 1976, the State Laboratory did not report any county with more than 2 rabies positive bats in a single year. During 1976, however, the State Laboratory reported 9 positive bats in Allen County and 5 positive bats in Wood County. The unusual clustering of positive bat rabies cases is evident in Allen and Wood Counties during 1976 as shown in Figure 2.

Wood County showed a 13-fold increase in number of bats submitted to the State Laboratory during 1976. Only

4 bats were submitted in 1975, while 52 bats were submitted during 1976 (51). A statistical analysis of Wood County's total 1976 submissions was performed. The ratio, 9.62% (5 of 52 bats submitted), was of borderline significance ($0.05 < p < 0.10$) when compared to the ratio of 3.16%, which is the ratio of rabid bats versus total bats submitted to the State Laboratory from 1956 through 1975.

The 5 rabies positive bats in Wood County originated from the same colony of M. lucifugus in a warehouse. Nine bats were found on the floor of the warehouse and were submitted to the State Laboratory. When 5 bats were found rabies positive by the FA test, State and Local Health Department officials requested and received permission from the Environmental Protection Agency and the Center for Disease Control to use DDT on a one-time basis for extermination of the colony.* Prior to use of DDT, the colony size was estimated by 2 health department people who counted the bats at dusk as the bats emerged for evening feeding. They estimated that 250 bats were in this colony. An unknown number of bats were killed by the DDT. Four bats were found dead in the warehouse and submitted to the State Laboratory. These 4 bats were FA test negative (47). For this colony of bats, 5 of 13 bats

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* This was the first time since being banned in 1972 that DDT was permitted for use.

(38.5%) submitted for rabies examination were positive. This was too small a sample to be statistically analyzed.

Allen County showed a 40-fold increase in number of bats submitted during 1976. Only 3 bats were submitted to the State Laboratory during 1975 from Allen County, while 122 bats were submitted during 1976 (51). The 9 rabies positive bats from 122 bats (7.38%) submitted in Allen County were significantly greater ($p < 0.05$) than the ratio (3.16%) for the period 1956 through 1975.

Allen County's 9 positive bats were from scattered locations in the county. Only 3 of the positive bats were identified. The bats identified were 2 E. fuscus, a colonial-living species, and 1 L. noctivagans, a solitary-living species. Colonies of origin for the 2 E. fuscus were unknown, so no colony with a known history of rabies could be sampled in Allen County.

A total of 500 bats (197 E. fuscus and 303 M. lucifugus) were captured for the present study. The date, location, species, and number of bats captured in each collection are shown in Table 5. Figure 3 is a map of the collection locations in Ohio.

A brain impression smear and a brown fat squash smear from each bat and the control mice were examined by the FA technique (Table 6). The brain impression smears from the intracerebrally inoculated positive control mice were FA

test positive. All other impression smears (brown fat from positive control mice, brains and brown fat from bats and negative control mice) were negative, though 5 bat brain impression smears showed a light, non-specific staining, difficult to distinguish from specific staining. Five duplicate brain impression smears from these bats were stained and these were negative. The duplicate slides did not show any non-specific staining. Suspensions of brain from these 5 bats and a brown fat suspension from 1 were intracerebrally inoculated into mice, as previously described. Ten mice were used for each group and observation continued for 28 days. Mice dying during this period were examined, but all were negative by the FA technique.

In addition, the first 136 bats examined by the FA test were divided into 13 groups of 10 bats and 1 group of 6 bats (Table 6). One-half the brain and 1/2 the brown fat from each bat of the group was pooled and intracerebrally inoculated into groups of 5 mice. All mice dying during the 28 day observation period were rabies negative by the FA technique.

The results of the FA test and mouse inoculations done in the present study would indicate that the prevalence rate of rabies in normal bats of Ohio is zero. This study's results were statistically analyzed by

comparing this study to the results of previous studies done in these species of bats in the United States, as summarized in Table 1. The rabies prevalence rate for E. fuscus (Table 1) is 1.33%. A prevalence rate of zero, as found in this study, is not significantly different ($p > 0.05$) from the estimated population rabies prevalence rate for E. fuscus. The estimated population prevalence rate for rabies in M. lucifugus is 0.20% (Table 1). This study's prevalence rate of zero for 303 M. lucifugus sampled is not significantly different ($p > 0.05$) from the estimated population rabies prevalence rate for M. lucifugus.

DISCUSSION

The ratio of rabies positive bats to total bats submitted during 1976 in Ohio was of borderline significance. Assuming the sampling was representative and using the 5% level of significance, it appears that a statewide epizootic of rabies in bats was not occurring in Ohio during 1976.

State and local health department officials revealed that most submissions of bats from Allen and Wood Counties were made after the first rabies positive bats were reported for each of these counties. News media and health department people publicized the State Laboratory's findings and alerted people to send in bats they found. The State Laboratory then received numerous bats that people may not have otherwise submitted (29,51,62).

In Wood County, where 5 of 13 bats (38.5%) from one colony were rabies positive, these extra submissions from other areas of the county may have masked a rabies outbreak occurring in this colony of bats. Consequently, Wood County's total ratio of 9.62% positive bats was of only borderline significance when compared to the ratio (3.16%) obtained in previous years for Ohio. The single colony sample size of 13 bats was too small to be statistically analyzed, but it appears that an outbreak of

rabies was occurring in this colony of bats during 1976.

A follow-up study of this colony could not be conducted since no bats returned to this roosting site after DDT was used. Many bats flew away when DDT was being sprayed into the colony and it is not known how many bats died later. Bats surviving would not be likely to return to this roosting site, but it is possible that these bats would seek out another colony. If these intruding bats were carrying rabies, it would be possible to transmit rabies to another colony and set up another nidus of bat rabies in Ohio or a surrounding state.

The finding of one rabies positive bat in Allen County during 1976 and its associated publicity appeared to be effective in alerting Allen County citizens to send in other bats they found. Among the bats sent in, the State Laboratory found additional rabies positive bats (51). The ratio of rabies positive bats (7.38%) was significantly greater than the ratio (3.16%) for previous years in Ohio. It is concluded that an outbreak of rabies existed in Allen County during 1976.

Only one colony of bats was sampled in Allen County for the present study (Table 5). Samples from additional colonies would have been useful, but the period within which this study was conducted did not permit time for surveying the county for additional collection locations.

Other counties bordering on Allen County may have also experienced a bat rabies outbreak. Sufficient bats were not submitted from these counties to be statistically analyzed.

The summary of previous surveys for prevalence of rabies in the normal bat population of the United States was presented in Table 1. Since only normal bats from these surveys were included in this table, the per cent positive bats is an estimate of the rabies prevalence in the bat population of the United States. It was assumed that Ohio's bat population is similar to the nation's bat population in this respect. The statistical analysis of the FA test results for the 500 bats captured in this study show that this study's results are within the 95% confidence interval of the rabies prevalence rate found for E. fuscus and M. lucifugus in previous studies.

Judging from these results, it is concluded that a bat rabies epizootic does not exist in Ohio at the present time, but outbreaks of bat rabies probably occurred in a colony of M. lucifugus in Wood County and an outbreak of bat rabies occurred in Allen County during 1976. The outbreak in Allen County seemed to be generalized over the entire county. Information from counties bordering Allen County was not sufficient for drawing conclusions.

Rabies positive bats diagnosed in Ohio have appeared to be associated with predominantly urban counties, rather than rural counties (Figure 1). Examples of counties showing this relation are: Hamilton County (Cincinnati), Montgomery County (Dayton), Franklin County (Columbus), Lorain and Cuyahoga Counties (Cleveland and Lorain), Summit County (Akron), Stark County (Canton), and Mahoning County (Youngstown). Exceptions to this observation are Lucas County (Toledo), which showed only 1 rabies positive bat in this time period, and Richland County (Mansfield), which had 10 rabies positive bats reported, but whose population doesn't match up with larger population centers.

The clustering of reported bat rabies cases around urban centers has 4 possible explanations:

1. Greater numbers of bats are present in these areas.
2. Higher rabies prevalence in bats of these areas.
3. Citizens in these areas were more alert to submitting bats for rabies examination.
4. A higher concentration of people giving a higher person : bat contact ratio.

Although roosting sites for bats are more plentiful for colonial-living bats in large cities, food supplies for bats would be less plentiful. Therefore, the first explanation is probably incorrect.

There is no reason to believe that rabies prevalence would be higher for bats living in large cities, but additional surveys would have to be done to rule this out.

The number of bats submitted depends on the alertness of the citizens to these creatures. Alertness to bats may be a quality developed by the people themselves, but it is more likely to be enhanced by news media releases and health departments stressing the public health importance of rabid bats. This was evident in Allen and Wood Counties during 1976, where submission of bats showed a dramatic increase when a rabies positive bat was diagnosed in the county (51).

The number of bats submitted from each county during 1975 is shown in Figure 4. If other years' submissions are similar, then more bats have been submitted from more populous counties of the state. It is obvious that a higher person : bat ratio exists in cities, so there are more people to observe and capture sick or injured bats. Also, there is a greater chance for bats to be trapped in houses in cities and thereby captured. With a greater number of bats submitted for rabies examination, there is a greater chance for more rabies positive bats being submitted, too. Richland County submitted 32 bats to the State Laboratory during 1975. If other years were similar to 1975, this could explain why Richland County has had

numbers of rabies positive bats comparable to more populous counties.

From this discussion, it appears that greater numbers of positive bats reported for certain counties of Ohio are related to a greater number of bats submitted for rabies examination. Counties with greater human populations and those counties where people are more aware of bat rabies are likely to be the counties from which more bats are submitted.

The rabies fluorescent antibody technique, as used in this study, was first developed and introduced by Goldwasser and Kissling in 1958 (31). Subsequently, state health laboratories began to use the technique on an experimental basis. Ohio started to use the FA technique in 1959. At that time, it was used in conjunction with Negri body examination and mouse inoculation. By 1963, the FA test completely replaced the Negri body examination for bats submitted to the Ohio Department of Health. Mouse inoculation is still used for verification (40).

In a summary of 24 studies conducted to evaluate the FA test for rabies diagnosis, 25,569 brains were tested by the mouse inoculation technique. Rabies positive brains were noted in 3049 cases. The FA test missed 87 of the 3049 positive cases diagnosed by mouse inoculation, giving the FA test a sensitivity of 97.1% (36). Specificity of

the FA test has been found to be 100% (1,37,38). Based on the validity of the FA test, as indicated in these studies, and the problems of logistics, time, and expense involved with testing specimens by mouse inoculation, the FA test method was chosen as the principal procedure for rabies diagnosis in this study.

The positive control brain impression smears used in this study showed small, almost dust-like, green fluorescence, typical of the FA test for rabies. Compared to the positive control slides used by the Ohio Department of Health, the positive control slides used in this study exhibited only about 30% as much fluorescence. This relatively small amount of fluorescence was attributed to the ERA strain (Jen Sal) of rabies virus used for inoculating control mice. One of the Ohio Department of Health's positive control slides was stained with the conjugate used in this study. This positive control slide showed fluorescence identical to slides stained with the Ohio Department of Health's conjugate, thus demonstrating that the conjugate used in this study was equivalent to the conjugate used by the State Laboratory.

Brown fat smears made from the positive control mice showed no fluorescence, indicating that rabies virus was not present in this tissue. The mice had been inoculated intracerebrally, so these results were expected.

Brain impression smears from 5 different bats gave questionable FA test results. Duplicate smears were stained and all were negative. It was concluded that the former group of slides showed non-specific staining, probably due to drying of conjugate on the smear. Mouse inoculation tests for these 5 bats were also negative, further supporting this conclusion.

Although the present study did not reveal any rabies positive bats, rabid bats do represent a serious public health hazard. Bites by insectivorous bats have accounted for 7 known human rabies cases in the United States (14, 15,26,32,34,56). Only 1 of these survived (32). In addition, 2 human deaths have been attributed to inhalation of rabies virus in bat infested caves (34,35).

The most recent case of human rabies caused by the bite of a rabid insectivorous bat occurred in Maryland during 1976. A woman was bitten by an E. fuscus, which was rabies positive by the FA test. She was administered human rabies immune globulin and a 21 day series of DEV. Two days after completing the series of vaccine, she became ill. Although the early diagnosis claimed this was a vaccine reaction, a later diagnosis established that the disease was rabies. The woman's condition deteriorated and she died 23 days after becoming ill (15).

Though human rabies cases due to bat bite have been documented, no pets are known to have developed rabies after contacting or being bitten by an insectivorous bat (3). No studies have been done to determine how frequently pets are bitten by bats. A retrospective study, encompassing Arizona, California, Colorado, and New Mexico, analyzed the circumstances under which rabid bats, diagnosed by the State Laboratories, were submitted. Workers looked for histories of dog, cat, or human contact and whether or not a bite was inflicted by the bat. These workers found that pets had contacted 29.8% of the rabies positive bats before the bats were submitted to the State Laboratories. It is unknown if these bats bit the pet during the contact. In the same study, humans had contact with 23.3% of the positive bats prior to submitting the bats to the State Laboratories. About half of these human contacts resulted in bites by the bat (17). Pets probably have a bite frequency, by rabies positive bats, equal to or greater than the bat bite frequency experienced by humans.

Pets and other carnivores are susceptible to bat rabies virus by intramuscular inoculation. This was demonstrated by inoculating cats, dogs, coyotes, raccoons, foxes, ringtails, skunks, and opossums with rabies virus isolated from a bat. Rabies deaths occurred in all the species except opossums (19,20).

Experimental transmission of rabies virus by bite from bats to other animals has also been achieved. In one case, bats transmitted rabies to mice by biting (9), while in another study, 2 foxes and 1 coyote developed rabies after being bitten by bats (18). Another study, similar to the latter study, did not demonstrate transmission of rabies to carnivores by bites of infected bats. In this study, bats were inoculated intracerebrally with rabies virus isolated from a bat of their own species. Each bat's saliva was checked before and after biting for presence of rabies virus. Although 31 bats had rabies positive brains at death, only 13 had rabies positive saliva at the time of biting carnivores. Of 56 carnivores bitten, none died. Only 1 cat developed rabies serum antibody which increased from < 1:5 to 1:13 (21).

Rabies infection by the oral route is also a potential source of exposure, since carnivores are known to prey on bats. Among carnivores that prey on bats are skunks, raccoons, and cats (4,22,25). Experimental infection of animals by ingestion of rabies virus-infected material has been shown. Mice were infected by ingestion of infected mouse brains in one study (52), while 6 of 18 skunks developed rabies after ingestion of a single rabies infected mouse by each skunk in another study. In this latter study, cats and ferrets did not develop rabies

after ingesting infected mice (8).

These studies demonstrate that rabies infected bats are a potential source of rabies infection for terrestrial animals under natural conditions. According to one epidemiologist, bats constitute the largest reservoir of rabies in the United States, both in terms of numbers and geographic distribution (63). Whether or not bats play a role in outbreaks of rabies in other wildlife is not yet known, though a correlation between fox rabies and caves harboring bats has been discussed (28). Additional studies are needed to describe, in more detail, what part bats play in the epidemiology of rabies.

With several bat transmitted rabies cases having occurred in humans, it is important to warn people of the potential danger involved with handling bats. People should be encouraged to submit sick bats to their health departments for rabies examination. Serious consideration should be given to eliminating bats from homes and school buildings by prohibiting their entrance and establishment of colonies, especially if young children play outside these buildings. Needless extermination of bats should not be encouraged, however, because these animals serve a valuable role in controlling insects.

SUMMARY

During 1976, Ohio reported 23 rabies positive bats, which was an unexpectedly large number. Statistical analysis of bat rabies cases in Ohio revealed that the per cent of rabies positive bats submitted to the Ohio Department of Health during 1976 was of borderline significance ($0.05 < p < 0.10$) when compared to the per cent positive for 1956 through 1975. Wood County's ratio of rabies positive bats versus total bats submitted (9.62%) during 1976 was also of borderline significance when compared to previous years in Ohio, while Allen County's ratio of rabies positive bats (7.38%) was significantly greater ($p < 0.05$) than the per cent of positive bats submitted from 1956 through 1975 in Ohio. Allen County's rabies positive bats were from scattered locations in the county, while 5 of 13 bats (38.5%) from a single colony in Wood County were rabies positive.

From 9 September 1976 to 27 June 1977, 500 bats (197 Eptesicus fuscus and 303 Myotis lucifugus) were collected from various locations in Ohio. All bats were negative for rabies in brain and brown fat when tested by the fluorescent antibody method.

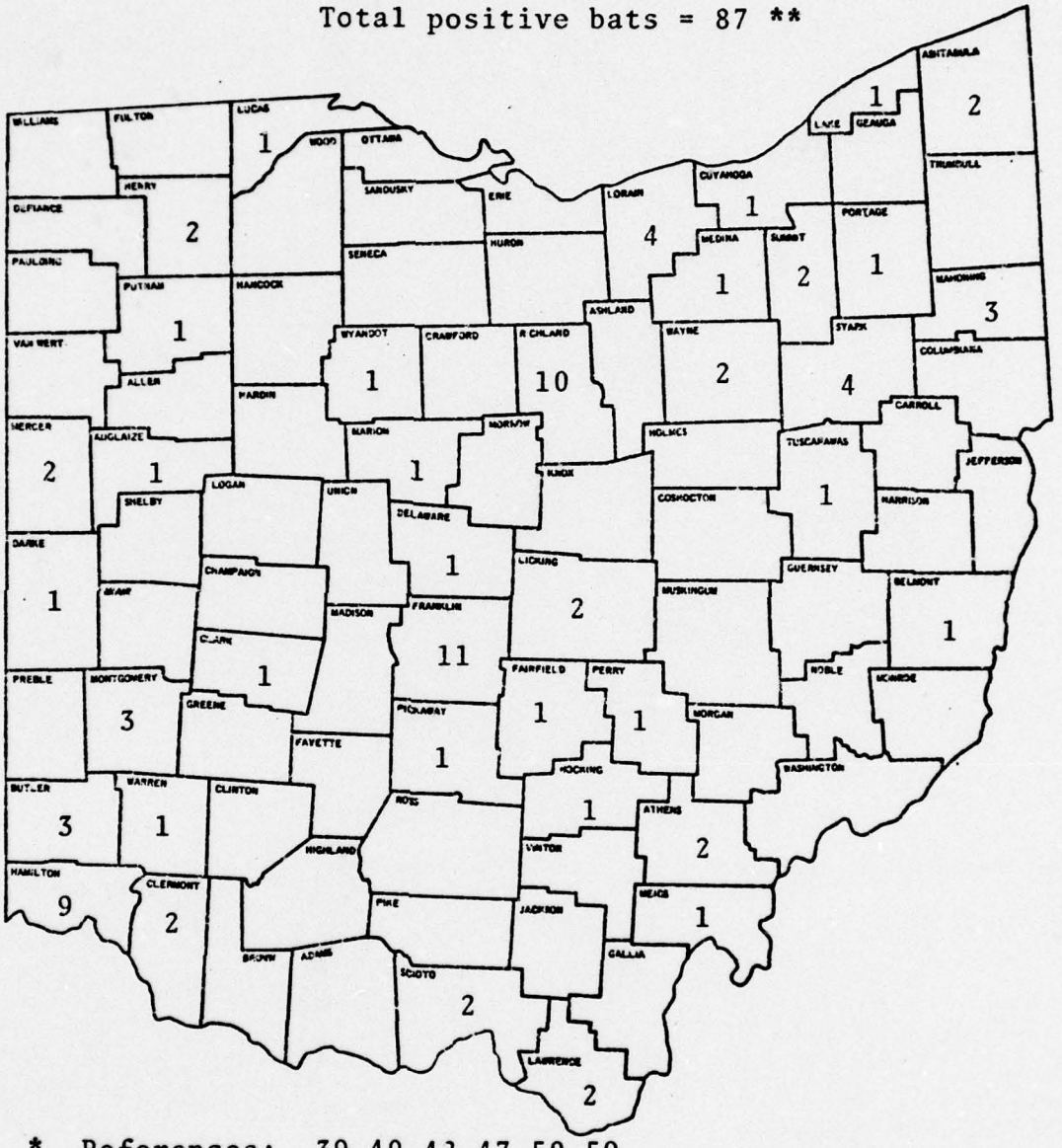
It was concluded that a statewide epizootic of bat rabies does not exist at the present time in Ohio, but a

local outbreak of bat rabies did occur in Allen County and probably also in a single colony of bats in Wood County during 1976.

FIGURE 1

RABIES POSITIVE BATS IN OHIO FROM 1956 THROUGH 1975 *

Total positive bats = 87 **



* References: 39,40,42,47,50,59

** Figure includes 2 rabies positive bats, 1 in Franklin County (59) and 1 in Stark County (39), which were found in surveys rather than in regular submissions to the State Laboratory.

FIGURE 2

RABIES POSITIVE BATS IN OHIO DURING 1976 *

Total positive bats = 23

* Reference: 47

FIGURE 3

LOCATION AND NUMBER OF BATS COLLECTED

E = E. fuscus

M = M. lucifugus

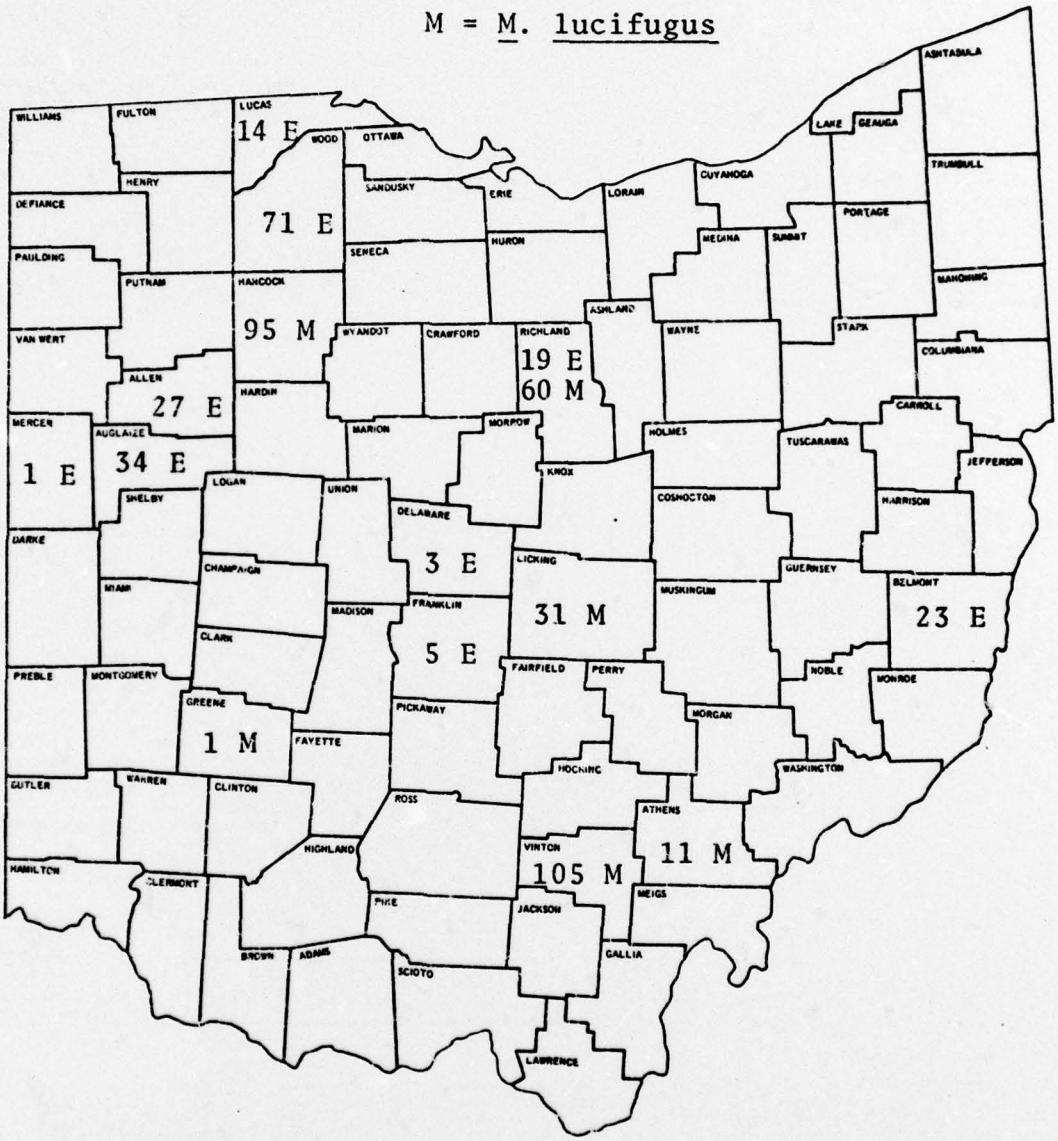


FIGURE 4

NUMBER OF BATS SUBMITTED TO THE OHIO DEPARTMENT OF HEALTH FOR RABIES EXAMINATION DURING 1975 BY COUNTY +

Total bats submitted = 274
Total positive bats = 8

+ Reference: 47

* Indicates rabies positive bat.

TABLE 1

BAT SPECIES AND RABIES PREVALENCE IN NORMAL BATS OF THE UNITED STATES

Bat Species	No. Coll.	Positive No.	Positive %	References
* Eptesicus fuscus **(Big brown bat)	450	6	1.33	5,23,24,30,59,60,61
* Myotis lucifugus (Little brown bat)	1005	2	0.20	5,23,30,61
Myotis austroriparius (Southeastern myotis)	2127	1	0.05	49,60
Myotis grisescens (Gray myotis)	281	1	0.36	49
Myotis velifer (Cave bat)	320	5	1.56	11,24,58
Tadarida brasiliensis mexicana (Mexican free-tailed bat)	3523	43	1.22	11,22,24,25,58
Tadarida brasiliensis cynocephala (Florida free-tailed bat)	1160	4	0.35	49,60
Tadarida brasiliensis (Free-tailed bat)	53	3	5.36	24
* Pipistrellus subflavus (Eastern pipistrelle)	383	2	0.52	11,23,49,58,60,61
Macrotus waterhousii californicus (California leaf-nosed bat)	84	0	0	24
* Nycticeius humeralis (Evening bat)	244	0	0	49,60
Antrozous pallidus (Desert pallid bat)	47	4	8.51	11,24
Dasypterus floridanus (Florida yellow bat)	717	20	2.79	49,60
Lasiurus seminolus (Seminole bat)	846	6	0.71	49,60
* Lasiurus borealis (Red bat)	183	5	2.73	11,30,49,60,61
Total	11,423	102	0.89	

* Bats native to Ohio.

** Common names in parentheses.

TABLE 2

BATS SUBMITTED TO THE OHIO DEPARTMENT OF HEALTH FOR RABIES EXAMINATION AND NUMBER OF RABIES POSITIVE BATS *

Year	Number Bats Submitted	Number Positive
1941-1953	0	0
1954	5	0
1955	1	1a
1956	4	2
1957	12	0
1958	34	2b
1959	37	3
1960	30	1
1961	103	4
1962	137	3
1963	118	4
1964	78	5
1965	74	6
1966	166	9
1967	c	7
1968	133	2
1969	107	8
1970	162	6
1971	331	6
1972	221	2
1973	214	3
1974	233	5
1975	274	8
1976	488	23

a Positive bat was from a sample of 63 normal E. fuscus captured (59).

b One positive bat was from a sample of 35 normal M. lucifugus captured (39).

c Figures not available.

* References: 16,40,42,44,51

TABLE 3

SPECIES AND NUMBER OF RABIES POSITIVE BATS
AND TOTAL BATS EXAMINED FOR RABIES IN OHIO BY SPECIES *

Species	Total Examined**	Number Positive
Eptesicus fuscus	1131	22
Myotis lucifugus	287	9
Lasiurus borealis	97	5
Lasiurus cinereus	16	3
Lasionycteris noctivagans	7	1
Pipistrellus subflavus	2	0
Myotis keeni	2	0
Unidentified bats	1297	47
Total	2839	87

* Includes 373 bats from surveys as listed by Schnurrenberger in 1964 (50). Other references: 41,51

** 1964 through 1968 omitted.

TABLE 4

COUNTY OF ORIGIN OF RABIES POSITIVE BATS
IN OHIO: 1955 TO 1976 **

1955 - Franklin*	1966 - Richland	1971 - Butler
	Auglaize	Hamilton
1956 - Henry (2)	Athens	Clark
	Fairfield	Franklin
1957 - 0	Ashtabula	Lake
	Lucas	Licking
1958 - Stark (2)	Mahoning	
	Wayne	1972 - Hamilton
1959 - Lorain	Mercer	Lawrence
Hamilton		
Licking	1967 - Darke	1973 - Mahoning
	Montgomery	Stark
1960 - Warren	Cuyahoga	Summit
	Hamilton	
1961 - Franklin (2)	Richland	1974 - Delaware
Montgomery	Perry	Pickaway
Richland	Lawrence	Putnam
		Richland
1962 - Athens	1968 - Hocking	Summit
Franklin	Mahoning	
Lorain		1975 - Richland (2)
	1969 - Richland (2)	Butler
1963 - Hamilton (2)	Stark	Franklin
Richland	Portage	Lorain
Franklin	Belmont	Marion
	Scioto	Scioto
1964 - Hamilton	Meigs	Wayne
Clermont	Tuscarawas	
Butler		1976 - Allen (9)
Ashtabula	1970 - Hamilton (2)	Wood (5)
Franklin	Mercer	Lucas (2)
	Wyandot	Ashtabula
1965 - Franklin (2)	Franklin	Fairfield
Lorain	Medina	Guernsey
Clermont		Hocking
Montgomery		Huron
Richland		Muskingum
		Putnam

* Counties with only 1 bat reported positive during year have no number following county name. More than 1 positive bat in a county is indicated by parentheses.

** References: 39,40,42,47,50,59

TABLE 5

NUMBER OF BATS COLLECTED BY DATE, LOCATION, SPECIES, AND METHOD OF CAPTURE

Date	County	Type of Building	Species	No. Coll.	Method of Capture
9 Sep 76	Athens	House attic	M. lucifugus	11ab	Hand
18 Nov 76	Franklin	Office bldg.	E. fuscus	1b	Hand
23 Nov 76	Franklin	Office bldg.	E. fuscus	1	Hand
22 Dec 76	Franklin	Office bldg.	E. fuscus	1b	Hand
2 Feb 77	Franklin	Office bldg.	E. fuscus	1	Hand
24 Feb 77	Franklin	Office bldg.	E. fuscus	1	Hand
26 Apr 77	Greene	Office bldg.	M. lucifugus	1	Hand
27 Apr 77	Wood	Church loft	E. fuscus	33	Hand
27 Apr 77	Wood	House attic	E. fuscus	3	Hand
6 May 77	Delaware	School attic	E. fuscus	3	Hand
7 May 77	Vinton	House attic	M. lucifugus	105	Hand
13 May 77	Richland	House attic	M. lucifugus	60	Hand
13 May 77	Richland	Office bldg.	E. fuscus	1	Hand
13 May 77	Richland	Barn	E. fuscus	18	Hand
25 May 77	Licking	House attic	M. lucifugus	31	Hand
8 Jun 77	Hancock	Garage	M. lucifugus	95	Net
9 Jun 77	Belmont	House attic	E. fuscus	23	Hand
13 Jun 77	Lucas	House	E. fuscus	14	Net
20 Jun 77	Auglaize	Nursing home	E. fuscus	34	Hand
20 Jun 77	Mercer	Rectory	E. fuscus	1	Hand
20 Jun 77	Allen	Barn	E. fuscus	27	Hand
27 Jun 77	Wood	Garage	E. fuscus	35	Hand

a Sample was captured by Dr. Jerry Svendson, Professor in Zoology, Ohio University, Athens, Ohio, and frozen in -20°C freezer for 2 weeks before being transferred to -70°C freezer.

b Bats were frozen at -70°C before being processed on 5 Apr 77.

TABLE 6

RESULTS OF FLUORESCENT ANTIBODY TESTS
AND MOUSE INOCULATION

Group	No.	No. FA* Test Positive		Mouse Inoc. No. Pos.
		Brain Impression	Brown Fat	
Field bats	500	0	0	
	14**			0
	6***			0
Normal mice	8	0	0	nd+
ERA++ inoc. mice	9	9	0	nd

* Fluorescent antibody

** 13 pools with 10 bats each and 1 pool with 6 bats.

*** 6 samples = 5 individual bat brain suspensions plus brown fat suspension from 1 bat.

\+ nd = not done

++ Jen Sal's ERA strain (Rabies Vaccine, Modified Live Virus, Porcine Tissue Culture Origin, High Cell Passage, Street Alabama Dufferin Strain).

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